

IN THE CLAIMS

1.- 19. (Cancelled)

20. (New) A method of amplifying a nucleic acid molecule comprising incubating an RNA template with a composition comprising (a) a buffer, (b) one or more proteins having reverse transcriptase activity and (c) at least one DNA polymerase;

under conditions which substantially relieve reverse-transcriptase mediated inhibition of DNA polymerase activity and which are sufficient to amplify a DNA molecule complementary to all or a portion of said RNA template,

wherein said buffer comprises glutamate in an amount effective to reduce said inhibition of said at least one DNA polymerase, and

wherein said DNA polymerase is selected from the group consisting of Tbr, Tru, Tli, Tac, Tih, Tfi, Kod, Bst, Sac, Sso, Poc, Pab, Mth, Pho, ES4, VENT, DEEPVENT, Tne, Tma, Taq, Pfu, Tth, Pwo, Tfl and combinations thereof.

21. (New) A method according to claim 20, wherein said composition comprises a first reverse transcriptase enzyme in which the reverse transcriptase activity resides in a single polypeptide and a second reverse transcriptase enzyme in which the reverse transcriptase activity resides in a dimeric or multimeric structure.

22. (New) The method according to claim 21 wherein said first reverse transcriptase enzyme is Moloney murine leukemia virus (M-MLV) reverse transcriptase or a derivative thereof having reduced RNase H activity and said second reverse transcriptase enzyme is AMV reverse transcriptase or a derivative thereof having reduced RNase H activity

23. (New) The method according to claim 20, wherein said composition comprises a first primer and a second primer;

wherein said first primer is suitable for facilitating synthesis of first strand cDNA from said RNA template, and wherein the combination of said first and said second primer is suitable for amplifying said first strand cDNA.

24. (New) The method according to claim 20, wherein the total glutamate concentration is from about 1 mM to about 500 mM.

25. (New) A method for accurately quantifying a nucleic acid molecule in an essentially sequence-independent manner comprising

incubating an RNA template with a composition comprising (a) a buffer, (b) one or more proteins having reverse transcriptase activity, (c) at least one DNA polymerase, and (d) a first primer and a second primer,

wherein said buffer comprises glutamate in an amount effective to reduce said inhibition of said at least one DNA polymerase,

wherein said first primer is suitable for facilitating synthesis of first strand cDNA from said RNA template, and wherein the combination of said first and said second primer is suitable for amplifying said first cDNA,

wherein said incubation is under conditions which substantially relieve reverse-transcriptase-mediated inhibition of DNA polymerase activity and which are sufficient to amplify a DNA molecule complementary to all or a portion of said RNA template,
and

wherein said DNA polymerase is selected from the group consisting of Tbr, Tru, Tli, Tac, Tih, Tfi, Kod, Bst, Sac, Sso, Poc, Pab, Mth, Pho, ES4, VENT, DEEPVENT, Tne, Tma, Taq, Pfu, Tth, Pwo, Tfl and combinations thereof.

26. (New) A method for the unbiased quantification of a nucleic acid molecule contained in a sample comprising

incubating an RNA template with a composition comprising (a) a buffer, (b) one or more proteins having reverse transcriptase activity, (c) at least one DNA polymerase, and (d) a first primer and a second primer,

wherein said buffer comprises glutamate in an amount effective to reduce said inhibition of said at least one DNA polymerase,

wherein said first primer is suitable for facilitating synthesis of first strand cDNA from said RNA template, and wherein the combination of said first and said second primer is suitable for amplifying said first strand cDNA,

wherein said incubation is under conditions which substantially relieve reverse-transcriptase-mediated inhibition of DNA polymerase activity and which are sufficient to amplify a DNA molecule complementary to all or a portion of said RNA template,
and

wherein said DNA polymerase is selected from the group consisting of Tbr, Tru, Tli, Tac, Tih, Tfi, Kod, Bst, Sac, Sso, Poc, Pab, Mth, Pho, ES4, VENT, DEEPVENT, Tne, Tma, Taq, Pfu, Tth, Pwo, Tfl and combinations thereof.

27. (New) A method according to claim 25, wherein said composition comprises a first reverse transcriptase enzyme in which the reverse transcriptase activity resides in a single polypeptide and a second reverse transcriptase enzyme in which the reverse transcriptase activity resides in a dimeric or multimeric structure.

28. (New) The method according to claim 27 wherein said first reverse transcriptase enzyme is Moloney murine leukemia virus (M-MLV) reverse transcriptase or a derivative thereof having reduced RNase H activity and said second reverse transcriptase enzyme is AMV reverse transcriptase or a derivative thereof having reduced RNase H activity.

29. (New) The method according to claim 26, wherein said composition comprises a first reverse transcriptase enzyme in which the reverse transcriptase activity resides in a single polypeptide and a second reverse transcriptase enzyme in which the reverse transcriptase activity resides in a dimeric or multimeric structure.

30. (New) The method according to claim 29 wherein said first reverse transcriptase enzyme is Moloney murine leukemia virus (M-MLV) reverse transcriptase or a derivative thereof having reduced RNase H activity and said second reverse transcriptase is AMV reverse transcriptase or a derivative thereof having reduced RNase H activity.

31. (New) The method according to claim 25, wherein said at least one glutamate compound is selected from the group consisting of glutamate salts of organic bases, alkali metal glutamate salts and alkaline earth metal glutamate salts.

32. (New) The method according to claim 26, wherein said at least one glutamate compound is selected from the group consisting of glutamate salts of organic bases, alkali metal glutamate salts and alkaline earth metal glutamate salts.

33. (New) The method according to claim 20, wherein said buffer further comprises an effective amount of an antifoam compound.

34. (New) The method according to claim 20, wherein said buffer further comprises a sulfur-containing compound and a potassium-containing compound.